radiographic technique. Indeed the amount of radioactivity we should find in the contaminating bacteria should represent at least one to several percent of the total radioactivity present in the tissues. In fact it should amount to even more since over 90% of ^{3}H RNA extracted from bacteria cultured in Ringer's solution and labelled for 3 h is ribosomial ^{3}H RNA. Now the rate of hybridization of ribosomial bacterial RNA with bacterial DNA is always very low. This means, to account for our results, the amount of labelled contaminating bacteria should be in such quantity that they could not be missed. It should be stressed that when frog auricles are bathed in a suspension of labelled bacteria[7] and not treated with antibiotics these bacteria can be easily detected by autoradiography even if they are in such small quantities as, for instance, 5 bacteria per auricle section. Moreover similar results are obtained when the labelling takes place in vivo or in vitro after an antibiotic treatment. Therefore the ^{3}H RNA extracted from the auricles of the infected animals has necessarily been synthesized by animal cells.

Our results show that the phenomenon we had previously observed when separate organs were dipped in a suspension of bacteria, can also occur when an animal suffers a general bacterial infection.

The implications are difficult to evaluate. It is, however, tempting to postulate that this penomenon might be linked to some immunological reactions as we know many different kinds of compounds can be antigenic. It would also be worth investigating whether this mechanism might not play some part in causing the pathological effects on the heart seen after such infections as scarlet fever and rheumatic fever.

Résumé. Des organes de plantes ou d'animaux mis dans une suspension bactérienne synthétisent du RNA bactérien. Ce phénomène que nous avons appelé «transcession» est dû au transfert de DNA spontanément cédé par les bactéries vivantes aux cellules des organismes supérieurs. Dans le présent travail, il est démontré que le même phénomène peut avoir lieu naturellement lorsqu'une grenouille est sujette à une infection bactérienne.

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Zone Electrophoretic Studies on Lactate Dehydrogenase Isoenzymes in South American Cichlids (Teleostei, Percomorphi)

Comparative biochemical data have often been suggested as sources of taxonomic information. Electrophoretic studies on animal proteins are increasingly used in confirming and supplementing taxonomic relationships derived from classical criteria[1–4]. Proteins which show little intraspecific variation, might be well suited for uncovering the phylogeny of closely related species. We have compared tissue-specific patterns of proteins in South American Cichlids (Teleostei, Percomorphi) by starch gel electrophoresis. Among various proteins investigated, the isoenzymes of lactate dehydrogenase (LDH; E.C.1.1.1.27) exhibited little intragenergic variation; however, striking differences became apparent by comparison of LDH isoenzyme patterns of species from different genera.

Mitochondria-free supernatant fractions of tissue homogenates were subjected to starch gel electrophoresis, using the vertical electrophoresis apparatus from Buchler Instruments (Fort Lee, N.J., USA). 12% starch gels (Connaught, starch-hydrolysed) were prepared in a continuous triple buffer system (ethylenediamine tetraacetic acid, boric acid, *tris*[5]), pH 8.6. Electrophoresis was conducted at 8 V/cm for 16 h. All preparations were done at 4 °C. LDH isoenzymes were visualized by incubation of gel slices at 30 °C for 30 min in 45 ml 0.2 *M tris*, pH 8.0, containing 120 mg NAD, 0.4 ml L-lactate (1 *M*), 1.2 mg PMS and 8 mg Nitro-BT. Blanks contained no lactate solution. Most animals which were used in this study, were bred at the Gesellschaft für Strahlenforschung mbH, Munich, and originated from commercial breeders.

LDH exists in multiple molecular forms in vertebrates[6]. The LDH polypeptides in teleostean fish are encoded in at least 3 codominant loci[7–9], and are designated A, B and E[7]. These polypeptides are usually expressed tissue-specifically because of differential activities of LDH genes, which code for polypeptides A, B and E. Four polypeptides associate to form enzymatically active tetrameric lactate dehydrogenases[10]. If more than one polypeptide type is synthetized in one cell, a series of heterotetrameric LDH isoenzymes may be formed by random association of different polypeptides[10]. However, the isoenzyme patterns in those fish-tissues where more than one LDH polypeptide is expressed, often deviate from the expected binominal distribution of homo- and heterotetrameric LDHs. Mainly the homopolymers are found[8].

Figure 1 shows a LDH-zymogram of 2 species of *Cichlasoma: C. citrinellum* and *C. spilurum*. The tissue-specific patterns of LDH isoenzymes are virtually identical in both cichlids. Skeletal muscle contains only LDH A_4, extracts from heart- and liver homogenates only LDH B_4. Brain tissues contain both A_4 and B_4 LDH. Traces of heteropolymers from A and B subunits are observed on the zymograms of this tissue at positions intermediate between the isoenzymes A_4 and B_4. Lactate dehydrogenase

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E_4 was only observed in homogenates from eyes. These extracts also contained isoenzymes A_4 and B_4, but only traces of heteropolymers at intermediate positions on the zymograms. Since whole eyes were used for preparation of homogenates, the 3 isoenzymes which are observed in extracts, might well be located in different types of cells.

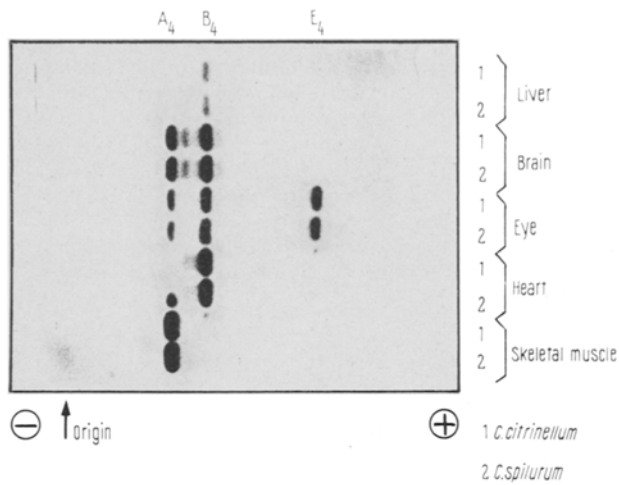

Fig. 1. Vertical starch gel electrophoresis of high speed supernatants of tissue homogenates from *Cichlasoma citrinellum* and *Cichlasoma spilurum*. Specific staining for LDH. The arrow marks the origin. Continuous buffer system: EDTA-*tris*-boric acid, pH 8.6, 8 V/cm, 16 h.

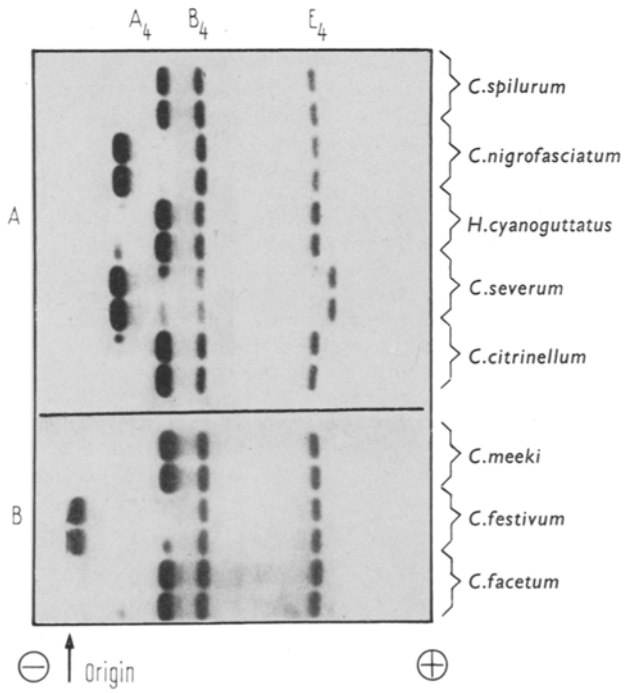

Fig. 2. Vertical starch gel electrophoresis of high speed supernatants of tissue homogenates from 7 *Cichlosoma* species and *Herichthys cyanoguttatus*. Conditions as Figure 1. Each slot contained extracts of a different animal. 35 µl of supernatant from eye-homogenate (25%) + 10 µl of supernatant from skeletal muscle homogenate (25%) were inserted in each slot in order to compare the homopolymeric LDHs A_4, B_4 and E_4. A and B were 2 different gels.

With respect to tissue-specific distribution of LDH isoenzymes, there was no difference in 17 species of cichlids from 7 genera. However, some differences were observed in the electrophoretic mobilities of isoenzymes A_4, B_4 and E_4.

Within the genus *Cichlasoma* the net charge of LDH B_4 was identical in all species (Figure 2), as judged by electrophoretic mobility of this isoenzyme. Similarly LDH E_4 exhibited identical net charge in all species except in *Cichlasoma severum* (Figure 2). 3 different phenotypes of LDH A_4, however, were observed in this genus. A_4 of *Cichlasoma festivum* was found close to the origin on gels. A_4 of *Cichlasoma severum* and *C. nigrofasciatum* was found intermediate between the origin and isoenzyme B_4 (Figure 2). A_4 of the species *Cichlasoma citrinellum*, *C. facetum*, *C. meeki* and *C. spilurum* was located more closely to LDH B_4.

In addition to 7 species of *Cichlasoma*, we have investigated the LDH isoenzyme patterns of cichlids of other genera, some of which exhibited striking similarities with the genus *Cichlasoma* in these biochemical phenotypes. Thus, the net charges of LDHs A_4, B_4 and E_4 of *Herichthys cyanoguttatus* were identical to those of *Cichlasoma citrinellum*, *C. meeki*, *C. facetum* and *C. spilurum* (Figure 2). *Herotilapia multispinosa*, *Pterophyllum scalare* and *Symphysodon aequifasciata* were indistinguishable in their LDH zymograms from *Cichlasoma nigrofasciatum* (Figure 3). *Cichlasoma nigrofasciatum* was also identical to 3 species of the genus *Aequidens*, except for LDH B_4 (Figure 3). Three species of *Apistogramma* differed from all other Cichlids in their LDHs B_4 and E_4, but were identical to *Cichlasoma citrinellum*, *C. spilurum*, *C. meeki*, *C. facetum* and *Herichthys cyanoguttatus* in LDH A_4 (Figure 3).

MARKERT and WHITT[11] pointed out that the selection pressure for maintaining the net charge of an isoenzyme of LDH in any particular species is very strong. The similarities in isoenzyme patterns of LDHs which are observed

[11] C. L. MARKERT and G. S. WHITT, Experientia *24*, 977 (1968).

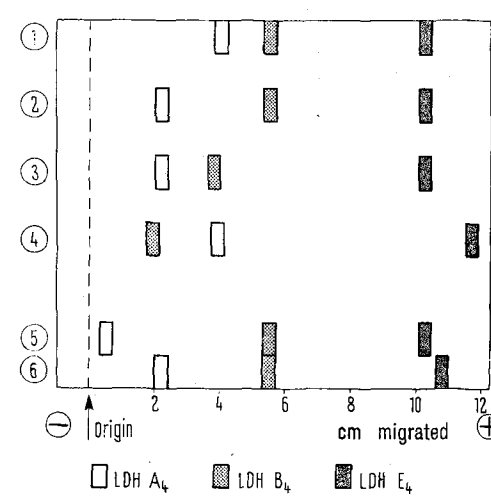

Fig. 3. Electrophoretic mobility of lactate dehydrogenases A_4, B_4 and E_4 in cichlids of different genera: 1. Phenotype of *Cichlasoma citrinellum*, *C. spilurum*, *C. meeki*, *C. facetum* and *Herichthys cyanoguttatus*. 2. *Herotilapia multispinosa*, *Pterophyllum scalare*, *Symphysodon aequifasciata* and *Cichlasoma nigrofsciatum*. 3. *Aequidens tetramerus*, *Ae. pulcher* and *Ae. curviceps*. 4. *Apistogramma pleurotaenia*, *Ap. ortmanni* and *agassizi*. 5. *Cichlasoma festivum*. 6. *Cichlasoma severum*.

in the cichlids might therefore reflect the degree of phylogenetic relatedness. Thus *Cichlasoma spilurum, C. facetum, C. meeki* and *C. citrinellum* might be related and *Herichthys cyanoguttatus* might be closely allied to them. This conclusion is confirmed by REGAN's work[12].

Similarly, *Herotilapia multispinosa, Symphysodon aquifasciata* and *Pterophyllum scalare* might by closely related to *Cichlasoma nigrofasciatum*. This is again in good agreement with REGAN[12]. According to REGAN's view *Herotilapia multispinosa* is closely related to *C. nigrofasciatum*, which is derived from facetum-type cichlids. *Pterophyllum scalare* and *Symphysodon aequifasciata* are however closely allied to *C. severum*, which like *C. nigrofasciatum*, is derived from facetum-type cichlids. This agrees with our results, if it is accepted that the different net charge of LDH E_4 in *C. severum* originates from a recent mutation in this species.

It would be premature to draw any further conclusions on the phylogeny of South American cichlids from a comparative investigation of three enzymes only, but it is substantiated by our results, that conveniently collected data on genetic variation, such as electrophoretic mobilities of specific proteins, might be an excellent tool for confirming and supplementing taxonomic relationships derived from classical criteria.

Zusammenfassung. Ein Vergleich der elektrophoretischen Mobilitäten der Laktatdehydrogenasen A_4 (Skelettmuskel), B_4 (Herz) und E_4 (Retina) zeigt bei 7 Arten der Gattung *Cichlasoma* weitgehende Übereinstimmungen. 10 Arten anderer Gattungen südamerikanischer Cichliden weisen, verglichen mit der Gattung *Cichlasoma*, zunehmende Divergenz der Isoenzymmuster auf. Es wird vermutet, dass sich hierin die phylogenetische Verwandtschaft der Gattungen widerspiegelt. Die auf Grund der Übereinstimmungen in LDH-Isoenzymmustern ermittelten phylogenetischen Zusammenhänge decken sich weitgehend mit den Beziehungen, die durch vergleichend-morphologische Untersuchungen aufgezeigt wurden. Das methodische Vorgehen scheint geeignet, taxonomische Beziehungen zu ergänzen, die durch klassische Kriterien dargelegt wurden[13].

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[14] The technical assistance of Mrs. EVELYNE PERRIARD is gratefully acknowledged.

PRO EXPERIMENTIS

Eine Methode zur Gewinnung von Ameisen-Spurpheromonen

Viele Ameisenarten kennzeichnen ihren Futterweg durch die Ausscheidung von Geruchsstoffen, die als Spurpheromone bezeichnet werden[1–6]. Das Spurpheromon von *Lasius fuliginosus* war Gegenstand eingehender Untersuchungen von HANGARTNER und BERNSTEIN[7] sowie von HANGARTNER[8], die unter anderem ergaben, dass sich die Substanz in der Rektalampulle sammelt und durch die Analöffnung ausgeschieden wird.

Das Problem der Isolierung dieses Naturstoffs lässt sich, je nach der Wahl des Ausgangsmaterials, auf vier verschiedene Weisen angehen. Das Ausgangsmaterial kann sein a) ein Ameisengesamtextrakt, b) ein Abdominalextrakt, c) der Inhalt der aus den Ameisen herauspräparierten Rektalampullen, d) die Gesamtheit der von den Ameisen auf dem Futterweg ausgeschiedenen Stoffe. Im folgenden wird eine Anlage beschrieben, welche erlaubt, die von den Ameisen einer Laboratoriumskolonie auf dem Futterweg ausgeschiedenen Stoffe laufend und ohne Störung der Tiere zu sammeln.

Die Anlage besteht aus einem Kunstnest und einer Reihe von Futterkästchen, von denen jedes über eine Vorrichtung zu erreichen ist, die als Eluierkammer bezeichnet werden soll. Figur 1 zeigt schematisch eine mögliche Anordnung dieser Bauelemente. Das Kunstnest ist in einem PVC-Behälter ($70 \times 45 \times 15$ cm) untergebracht, dessen Boden mit einer 5 mm hohen Gipsschicht belegt ist. Die Ränder sind mit Paraffinöl eingestrichen, um die Ameisen am Entweichen zu hindern. Eine Scheidewand unterteilt den Behälter in den Nestraum N und die Arena A. Nest

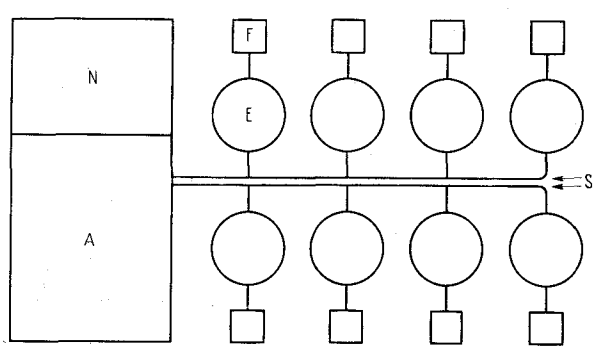

Fig. 1. N, Nestraum; A, Arena; E, Eluierkammer; F, Futterkästchen; S, Silicongummischläuche.

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